

INBREEDING DEPRESSION IN AUSTRALIAN BUTTERFLIES: SOME IMPLICATIONS FOR CONSERVATION

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Inbreeding depression in butterflies is manifested in many ways, including egg inviability, retarded larval development and premature mortality, failure to complete pupation and increased mortality during the pupal stage and at eclosion, and reduction in size of adults with an associated loss of vigour and reduced fecundity. Of the species examined, inbreeding depression was most severe in Papilionidae, especially the troidines *Cressida cressida* (Fabricius) and *Ornithoptera richmondia* (Gray), and in the papilionine *Papilio aegaeus* (Donovan). In *C. cressida*, hatching rates from matings between full siblings averaged about 40% and very few larvae survived to adulthood, whereas hatching rates from presumed outbred matings were nearly 100%. Hatching rates from full sibling matings in *O. richmondia* and *P. aegaeus* were about 70% and declined sharply as the coefficient of inbreeding was increased. In other species tested, papilionids *Chilasa anactus* (W.S. Macleay) and *Graphium macleayanum* (Leach), the pierid *Delias nigrina* (Fabricius) and the nymphalids *Danaus affinis* (Fabricius) and *Tellervo zoilus* (Fabricius), inbreeding depression was either not detected or was not apparent until the inbreeding coefficient exceeded 0.5. Inbreeding depression may be more severe in wide ranging species with an open population structure, and it is evident that given the severity of inbreeding depression recorded in troidine species such as *Ornithoptera richmondia*, it is necessary to conserve adequate habitat to maintain viable populations. Evidence is presented of inbreeding in wild *O. richmondia*. □ *Butterflies, conservation, captive breeding, inbreeding depression, Papilionidae.*

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Deleterious effects of inbreeding in organisms as diverse as white mice, *Drosophila* and cultivated maize are well known (Falconer, 1981; Wright, 1977). Typically, reproductive processes and early development are most affected (Frankel & Soulé, 1981). In holometabolous insects, ways in which inbreeding depression can be expressed include reduction in size of adult progeny and reduced fecundity, retarded larval development and failure to pupate or eclose successfully. More severe cases may suffer premature death in larval stages or in the egg, sometimes very early in embryogenesis (Clayton et al., 1957; Wallace & Madden, 1965; Wright, 1977).

Despite extensive breeding programmes for scientific and commercial purposes, inbreeding depression has seldom been reported in butterflies. In a survey of case histories of extinctions, Ehrlich (1983) concluded that stochastic demographic factors caused extinction long before inbreeding would have had a chance to take effect. Ehrlich's sample may have been unrepresentative since he considered mostly localised species from relatively closed populations (Ehrlich, 1965; Gilbert & Singer, 1973) inhabiting temperate, highly seasonal environ-

ments and frequently subject to catastrophic mortality due to adverse weather (Ehrlich et al., 1972, 1980).

In general, inbreeding depression would most likely be severe in wide ranging species which habitually outbreed, either because their open population structure has allowed accumulation of deleterious alleles or because they have been selected for a mating system which maximises outbreeding because of the effects of inbreeding. In either case, in the short time allowed to adjust to human alteration of the environment, species subject to severe inbreeding depression will need on average larger minimum viable populations. That means larger areas of suitable habitat. They will also generally be more sensitive to habitat fragmentation. Although the degree to which they are affected will depend on their ability to disperse, especially across tracts of unfavourable habitat. Inbreeding depression may also tend to be a more significant factor in extinctions of tropical species, many of which habitually exist at low population levels and may often pass through prolonged bottlenecks (Owen, 1971; Wolda, 1978) but are probably not normally sub-

ject to catastrophic density independent mortality.

I here investigate the effects of inbreeding in laboratory crosses in eight species of tropical butterflies. Species chosen are common and widespread but knowledge of their inbreeding susceptibilities may suggest possible risks in related vulnerable species.

MATERIALS AND METHODS

Species studied were *Ornithoptera richmondia* (Gray), *Cressida cressida* (Fabricius), *Papilio aegeus* Donovan, *Chilasa anactus* (W.S. Macleay), *Graphium macleayanum* (Leach) (all Papilionidae), *Delias nigrina* (Fabricius) (Pieridae), *Danaus affinis* (Fabricius) and *Tellervo zollus* (Fabricius) (Nymphalidae), and were selected because wild material was readily available for captive breeding and, in most cases, data were available on population structure based on mark-release-recapture (MRR) data.

For all species, 5-10 mated females were collected from the wild and allowed to oviposit on their host plants in a 2.5 x 3 x 4m flight cage. These females had presumably mated with an unrelated male. Eggs were collected from each female as they were laid and 10-20 larvae were reared to adulthood on appropriate hostplant. From these progeny, at least one brother-sister mating was arranged among the progeny of each original female. Matings were allowed to take place in the flight cage. That avoided hand pairing which might have interfered with normal reproductive processes. The first 50 or 100 eggs produced by each consanguineously mated female were collected as they were laid, and the hatching rate was monitored. Any eggs which did not hatch or show other external signs of development (like darkening after sclerotization of head capsule) were dissected and examined microscopically to check for an embryo, indicating the egg was fertile. A subsample of hatching eggs (usually 50-100) including roughly equal numbers from each of the inbred matings was reared to adulthood and survival recorded. Forewing lengths of surviving adults were measured and compared with the midparent value.

More extensive data were obtained for *Cressida cressida* and *O. richmondia*. For *C. cressida*, initial stock was provided by 10 wild caught females (presumed outbred). From their progeny four inbred sib-sib matings each were arranged, and 100 eggs collected from each female, in total 400 eggs. All hatching larvae were raised until

they died or pupated and developmental times were recorded. Successfully eclosing adults (with inbreeding coefficient $F=0.25$) from six of the ten original lines were mated: 1, with their sibs; and 2, with the inbred progeny from other lines. Hatching rates of samples of 100 eggs per female were recorded and all larvae were raised as far as possible. From resulting progeny ($F=0.44$), three unrelated pairs of siblings were mated with each other and hatching rates recorded. Two pairs from different lines were crossed and hatching rates recorded. Similar data were obtained for *O. richmondia*, based on an initial stock of five wild females, five inbred pairs per generation, and samples of 50 eggs.

For *Tellervo zollus*, the offspring of four females were inbred for four generations, selecting one pair from each line each generation. Hatching rates, larval survival and developmental rates, and adult sizes were recorded as above. Also, the lifetime fecundity of subsamples of females was recorded.

RESULTS

1. *Cressida cressida*: Hatching rates of eggs laid by wild females were all high, ranging from 97-100% ($n=100$), as was larval survivorship (89%, $n=100$) (Fig. 1). Hatching rates of eggs with an inbreeding coefficient ($F_{\text{offspring}}$) of 0.25 ranged from zero to 79% with a mean of 43%. Larvae which hatched frequently showed retarded growth (Fig. 2), particularly in early instars, and survivorship to adult stage was low, averaging 7.3% of eggs hatching. Developmental times ranged from 21-47 days (mean, 33 days), considerably longer than the normal develop-

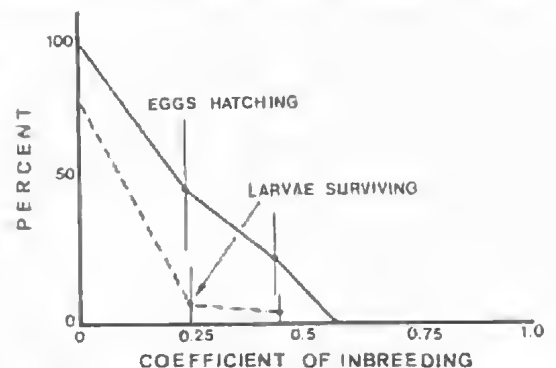


FIG. 1. Effects of increasing levels of inbreeding on hatching rate of eggs (solid line), and survival of hatching larvae (dashed line) in *Cressida cressida*. Vertical bars indicate standard deviations.

TABLE 1. Hatching rates and larval survivorship from inbred matings in five butterfly species. In all cases coefficient of inbreeding equals 0.25.

Species	<i>Papilia aëgeus</i>	<i>Chilasa anactus</i>	<i>Graphium macleayanum</i>	<i>Delias nigrina</i>	<i>Danaus affinis</i>
Total number of inbred matings	10	5	6	8	5
Mean hatching rate & total [n]	69%[500]	97%[250]	96%[300]	99%[500]	98%[250]
Mean larval survivorship [n]	32%[50]	87%[60]	92%[50]	82%[120]	85%[45]



FIG. 2. An inbred cohort of *Cressida cressida* larvae which hatched on the same day. The fourth instar (at right) had developed normally while its three siblings were still in the second instar.

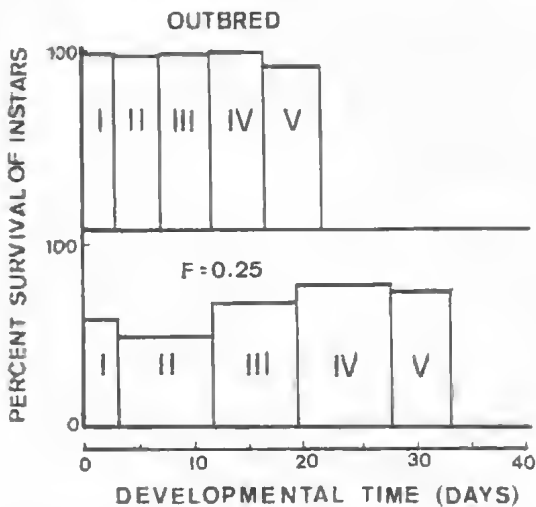


FIG. 3. Larval survival and rate of development by instar in inbred ($F=0.25$) and outbred *Cressida cressida*.

mental time of 21 days in outbred larvae (Fig. 3). In total, of 125 adults bred from 4000 eggs, seven were obviously stunted but the remainder were of normal size, robust and healthy. When these F1 adults were mated with their siblings (6 unrelated pairs, $F_{\text{offspring}} = 0.44$), hatching rates were very low, ranging from zero to 38% (mean=22%), and

larval survivorship was 5.9% of hatching eggs (13 adults from 600 eggs). When these F2 adults were mated (2 pairs, $F_{\text{offspring}} = 0.58$ with siblings the hatching rate of eggs was zero. When the inbred F1 adults were crossed out, the hatching rate of eggs was nearly normal (95%, $n=500$, 5 pairs). The survival of larvae was not monitored for these matings.

2. *Ornithoptera richmondia*: Slightly less spectacular but nevertheless severe inbreeding effects were recorded in *O. richmondia* (Fig. 4). Hatching rates of presumed outbred eggs from wild females were 97% ($n=500$) and larval survival was 82% ($n=100$). Hatching rates of eggs from sibling crosses ($F_{\text{offspring}} = 0.25$) ranged from 62 to 84% (mean = 74%). Larval survival was low, with only 11% of hatching eggs reaching adulthood. In total, 20 adults were reared from 250 eggs. Larval development was retarded, particularly in the later instars, and developmental time ranged from 25 to 52 days (mean 36 days), compared with a normal time of 27 days for outbred larvae. When the progeny of these crosses were mated ($F_{\text{offspring}} = 0.25$), hatching rates of eggs ranged from 42 to 59% (mean = 51%). Larvae hatching all contracted a probable viral infection in the fourth instar and died but this may not have been a direct effect of inbreeding.

3. *Other species*: In other papilionids, inbreeding depression as expressed by hatching rates of eggs from F1 sib-sib matings ($F_{\text{offspring}} = 0.25$) was most severe in *Papilio aëgeus* (Hatching rate 70%). Survivorship of hatching larvae was low (32%). Neither hatching rates nor larval survival rates in *Graphium macleayanum* and *Chilasa anactus* were significantly different from outbred matings. Similarly, by the same criteria, there was no evidence of inbreeding depression in either *Delias nigrina* or *Danaus affinis* (Table 1). In no cases were there any significant differences in size between parents and inbred offspring.

4. *Tellervo zoilus* (Fig. 6): There was no detectable reduction in hatching rates of eggs regardless of the degree of inbreeding ($n=672$), and larvae experienced high survivorship ($n=134$). However, adults with an inbreeding coefficient 0.5 and above characteristically were 10-20%

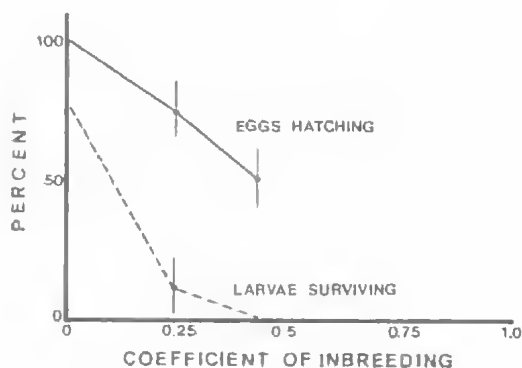


FIG. 4. Effects of increasing levels of inbreeding on hatching rates of eggs (solid line), and survival of hatching larvae (dashed line) in *Ornithoptera richmondia*. Vertical bars indicate standard deviations.

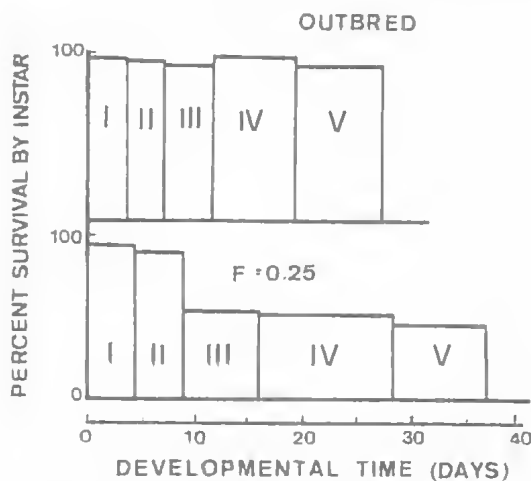


FIG. 5. Larval survival and rate of development by instar in inbred ($F=0.25$) and outbred *Ornithoptera richmondia*.

smaller (based on forewing length) than the original wild stock, ($n_{\text{inbred}} = 24$, $n_{\text{outbred}} = 40$ $t=3.8$, $p<0.001$). Corresponding with this, fecundity of females also dropped. There are no data on the normal relationship between fecundity and winglength but the fall in fecundity was of an order which suggested that no additional effects beyond the reduction in size were in operation.

5. Inbreeding in nature: For all species showing severe inbreeding depression in the ovum, large numbers of eggs were collected from wild females. In almost all cases, the natural hatching rate was close to 100%. However, in February 1987, three out of eleven wild female *O. richmondia* laid eggs with hatching rates ranging from

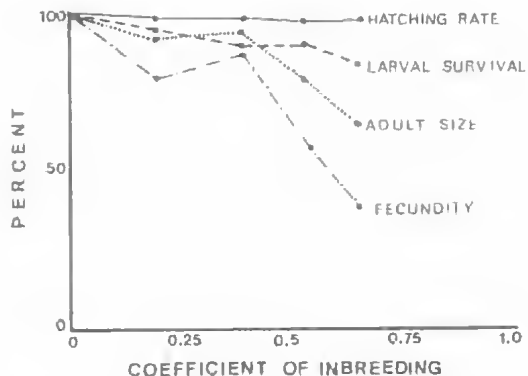


FIG. 6. Effects of increasing levels of inbreeding on hatching rate of eggs, larval survivorship, size and fecundity in *Tellervo zoihus*. Figures expressed as a percentage of outbred values.

64-76%. Hatching rates of eggs laid by the other eight individuals were normal (96-100%) and it seems most likely that low egg viability was a result of inbreeding as the individuals concerned were relatively young with fat body in good condition, as was determined by dissecting them after they had laid 50 eggs.

DISCUSSION

Although inbreeding depression has been reported in captive populations of various Lepidoptera (Norris, 1936; Waldbauer & Sternberg, 1978), effects as severe as those reported here have never previously been recorded in butterflies even though extensive breeding experiments have been conducted on several papilionid species (Clarke, 1972; Lederhouse & Schriber, 1984). Whether inbreeding depression was low in those species or not revealed by the breeding programme or was simply overlooked or ignored is not clear. In the only study of an endangered species which has addressed the possibility of inbreeding depression, Dempster & Hall (1980) found no evidence of reduced egg viability in the wild even though they were studying a small and dwindling population.

By contrast, this study indicates that in three species examined, inbreeding depression is potentially severe. Notably, these three are all papilionids, probably with very open population structures, as determined by MRR programmes (Orr, 1988). Inbreeding depression was not detected in *Graphium macleayanum* or *Danaus affinis*, in which dispersal is more limited. However, there is no clear pattern or susceptibility,

since in neither was there evidence of strong inbreeding depression in *Chilasa anactus* or *Delias nigrina*, both of which probably have fairly open population structures. However, as the continued inbreeding of *Tellervo zoilus* shows, most species will suffer deleterious effects if the inbreeding coefficient is sufficiently high.

Although these results cannot validly be extrapolated to other species, they suggest that certain papilionid groups, especially Troidini, may be highly susceptible to inbreeding. The IUCN Red Data Book on Papilionidae (Collins & Morris, 1985) lists 4 species as endangered, 23 species as vulnerable and 59 species as indeterminate, rare or requiring further monitoring. These include some of the most spectacular and distinctive species. With 24% of the world fauna (including *O. richmondia*) on the list, troidines are particularly well represented. If levels of inbreeding depression recorded in *C. cressida* or *O. richmondia* were in any way typical of the tribe then the actual risk to these listed species is probably even greater than assessed. In particular, captive breeding programmes (see New, 1991), frequently suggested as part of a species conservation strategy, may be doomed (see Martin, 1975; May, 1980).

Any conservation programme must ultimately aim at preserving a self-sustaining gene pool, which in turn must lead to the protection of minimum areas of habitat. As part of the general programme to conserve papilionid butterflies in particular, and probably all butterflies, the degree of susceptibility to inbreeding of all species under consideration must be assessed. Species which have formerly been widely distributed and whose habitat has been reduced and fragmented may be most at risk. Particularly if they are unwilling to fly across unfavourable habitat, but all species are potentially at risk if their population levels diminish to low levels for many generations (Frankel & Soulé, 1981).

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